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**Title:** Formulation of Soy-Based RTE Foods Influences Radiation Sensitivity of  
*Listeria monocytogenes* and Postirradiation Product Sensory Properties

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**FORMULATION OF SOY-BASED RTE FOODS INFLUENCES  
RADIATION SENSITIVITY OF *LISTERIA*  
*MONOCYTOGENES* AND POSTIRRADIATION  
PRODUCT SENSORY PROPERTIES<sup>1</sup>**

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**ABSTRACT**

*Ionizing radiation can eliminate pathogenic bacteria from ready-to-eat (RTE) food products. An outbreak strain of L. monocytogenes was irradiated after inoculation onto (1) three meatless, soy-based frankfurter products ("Soy1", "Soy2", "Soy3"), (2) soy-based tofu ("Tofu") and (3) a beef frankfurter product ("Beef"). The  $D_{10}$  was significantly influenced by the substrate: Beef (0.622 kGy) = Tofu (0.622 kGy) < Soy2 (0.680 kGy) = Soy3 (0.695 kGy) < Soy1 (0.761 kGy). The antioxidant strength of the products also varied significantly, but was not correlated with the  $D_{10}$  values obtained. To determine the sensory impact of irradiation, the products were treated with 1.5 kGy or 3.2 kGy, doses equivalent to 1.9-2.4 or 4.2-5.1  $\log_{10}$  reductions, respectively. These doses significantly decreased redness in Beef and Soy2, and significantly increased redness in Soy3. The maximum shear force of Beef and Soy1 was significantly decreased following irradiation. Product formulation was found to be key in determining the product response to irradiation.*

<sup>1</sup>Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

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## INTRODUCTION

The foodborne pathogen *Listeria monocytogenes* is capable of growth in high-salt environments, and refrigeration temperatures (Smith 1996). This pathogen has caused repeated outbreaks of foodborne illness and numerous recalls of frankfurters and other processed meat products (Anon. 1998; Barnes *et al.* 1989). Because listeriosis has a high frequency of mortality (~20%) among at-risk populations, *L. monocytogenes* is subject to zero-tolerance regulation in ready-to-eat (RTE) meat products in the United States (Mead *et al.* 1999; USDA 1989).

Ionizing radiation can effectively eliminate *L. monocytogenes* from a variety of food products, including frankfurters (Sommers *et al.* 2001). The pathogen's  $D_{10}$  value (amount of radiation necessary to reduce the population by 90%) can be influenced by the composition of the suspending food product (Niemira *et al.* 2002; Sommers and Thayer 2000). Frankfurter formulations can include vegetable-based ingredients such as soy protein concentrate (SPC) (Ockerman *et al.* 1989). Ingredients which have a high antioxidant capacity, such as SPC, can sometimes scavenge the radiolytic compounds produced during irradiation processing, thereby protecting bacteria and increasing the doses needed to achieve target kill levels (Ho *et al.* 1995; Sharma *et al.* 2000). A recent study of beef bologna formulations amended with up to 3.5% SPC showed that the dose required to achieve a 5-log<sub>10</sub> reduction of *L. monocytogenes* was significantly higher in formulations with greater antioxidant strength (Sommers *et al.* 2001). Although meatless, soy-based frankfurters present many of the same opportunities for postprocess contamination as meat-based frankfurters, studies of the  $D_{10}$  of *L. monocytogenes* in soy-based frankfurters are lacking.

The objectives of this study were (1) to determine the  $D_{10}$  of an outbreak strain of *L. monocytogenes* inoculated on the surface of commercial soy-based frankfurters, (2) to determine the effect of key doses of ionizing radiation on the color and texture of soy-based frankfurters.

## MATERIALS AND METHODS

### Products

Three types of soy-based frankfurter products were obtained in bulk from local markets. The three brands were designated Soy1, Soy2 and Soy3. Tofu (a soy-based emulsion-type food) and an all-beef frankfurter were included in all comparisons (Table 1). These products were designated Tofu and Beef, respectively. Before microbiological studies using these products were begun, the products were sterilized with gamma radiation using the method of Thayer *et al.* (1995). With the commercial packaging intact, the products were overpacked in laminated plastic/aluminum foil bags, vacuum sealed, and stored at -30C until given

IRRADIATION OF *L. MONO.* ON SOY FRANKFURTERSTABLE 1.  
DESCRIPTION OF COMMERCIAL READY-TO-EAT FOODS

Product	Unit Weight (g) <sup>1</sup>	Composition <sup>2</sup>	Comments
Beef	56	Beef, water, corn syrup, salt, potassium lactate, flavoring, sodium phosphate, ascorbic acid (vitamin C), sodium nitrate, extractives of paprika	Red, smooth
Soy1	52	Water, isolated soy protein, vital wheat gluten, expeller pressed canola oil, hydrolyzed corn and soy protein, salt, natural flavor, yeast extract, carrageenan (from seaweed), organic evaporated cane juice, spices, onion powder, garlic powder, natural liquid smoke, vitamin B1, vitamin B12, pantothenic acid, iron oxide, iron, zinc, magnesium, potassium.	Pink, smooth
Soy2	40	Water, tofu (Organically grown soybeans, water, magnesium chloride), textured soy protein concentrate, soy oil, soy protein isolate, tapioca starch, salt, natural vegetable flavors, spices, vegetable gums, malt extract.	Greenish yellow, colored flecks (spices)
Soy3	42	Water, soy protein isolate, wheat gluten, evaporated cane juice, salt, yeast extract, soy sauce (water, soybeans, wheat, salt), granulated garlic, carrageenan, spice extract, natural flavors from vegetable sources, vegetable gum, natural smoke flavor.	Pink, smooth
Tofu	43	Water, soybeans, isolated soy protein, gluconolactone	White, rubbery

<sup>1</sup>Weight of individual frankfurter links. In the case of Tofu, weight is of individual section.<sup>2</sup>As described in product ingredient list.

a sterilization treatment of 42 kGy, delivered at -30C. The irradiated, frozen products were stored at -30C until used. Prior studies have demonstrated that such treatments do not significantly alter the nutritional and chemical characteristics of foods (Thayer *et al.* 1995). The sterility of the products was confirmed prior to experimental use. To sample for background microflora, individual frankfurters were placed into a No. 400 stomacher bag (Tekmar, Inc., Cincinnati, OH). The weight of the individual frankfurters ranged from 40 g to 56 g among the commercial frankfurter brands, but was consistent within each brand. The block of

tofu was sectioned with a sterile knife into pieces weighing 43 g (Table 1). A single piece of tofu was bagged as described. Sterile Butterfield's phosphate buffer (BPB, Applied Research Institute, Newtown, CT), pH 7.2, was added to the stomacher bag, and the products were palpitated for 2 min to obtain a surface wash. The wash was serially diluted with BPB and plated on tryptic soy agar (TSA, Difco, Detroit, MI). Plates were incubated at 37C, and inspected after 24 and 48 h. Background microflora was found to be less than 10 cfu/g material for all products.

### **Microorganism**

An outbreak strain of *Listeria monocytogenes* associated with frankfurters (H7762, Center for Disease Control and Prevention, Atlanta, GA) was maintained on 50% glycerol at -70C. A frozen culture was regrown in tryptic soy broth (TSB, Difco, Detroit, MI) for 16 h at 37C with agitation and streaked onto Palcam agar (Difco, Detroit, MI). This was incubated at 37C for 48 h to form single colonies. These colonies were used to inoculate fresh TSB for each experiment, grown for 16 h at 37C with agitation. The cell density of the starting inoculum was determined by serial dilution with sterile BPB and pour plating with TSA. The cell density was typically  $10^9$  cfu/mL.

The products were aseptically opened in preparation for inoculation. In the case of the frankfurter products, a single frankfurter was placed into a No. 400 stomacher bag (Tekmar, Inc., Cincinnati, OH). The weight of the individual frankfurters ranged from 40 g to 56 g among the commercial frankfurter brands, but was consistent within each brand. The block of tofu was sectioned with a sterile knife into pieces weighing 43 g (Table 1). A single piece of tofu was bagged as described. A 200  $\mu$ L aliquot of *L. monocytogenes* culture was added to each sample bag. The bags were individually vacuum-sealed, and stored at 2C until irradiation, typically 60-90 min.

### **Irradiation**

The inoculated samples were treated with 0.0 (control), 0.5, 1.0, 1.5, 2.0 or 3.0 kGy. Each product was irradiated in three separate trials, using separately prepared sets of samples. These samples were irradiated concurrently using a Lockheed-Georgia (Manetta, GA) cesium-137 self-contained gamma radiation source, with a dose rate of 0.098 kGy/min. The temperature of the samples was held at 2C during irradiation by injection of gas-phase liquid nitrogen. Alanine pellets (Bruker, Inc. Billarna, MA) were used for dosimetry. The pellets were read on a Bruker EMS 104 EPR analyzer and compared with a previously determined standard curve. Actual dose was typically within 5% of the nominal dose.

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### Sampling

After irradiation, the samples were returned to refrigerated storage (2°C) until microbiological sampling, typically 30-60 min. The bagged samples were aseptically opened. Sterile BPB (100 mL) was added to the stomacher bag, and agitated for 60 s. A 1 mL sample was withdrawn for serial dilution with sterile BPB. Pour plating with TSA was used to determine the surviving bacterial population. Three pour plates per dilution were incubated for 48 h at 37°C and counted with an automatic plate counter. The data for each isolate were normalized against the control and plotted as the  $\log_{10}$  reduction using the nominal doses. The data from the three replications were pooled, and the slopes of the individual survivor curves were calculated by linear regression using a computer graphics program (SigmaPlot 5.0, SPSS Inc., Chicago, IL).  $D_{10}$  value (the radiation dose necessary to inactivate 90% of the population) was calculated by taking the negative reciprocal of the survivor curve slope (QuattroPro, Corel Corp. Ottawa, Ontario, Canada).

### Antioxidant Power

Nonirradiated samples were evaluated for antioxidant power using the ferric reducing/antioxidant power (FRAP) assay (Benzie and Strain 1999). A single frankfurter or piece of tofu was placed in a No. 400 stomacher bag with 100 mL BPB. The bag was stomached at high speed for 6 min to make a homogenized suspension. Separate aliquots (1 mL) were dispensed into 6 separate microcentrifuge tubes and spun to pelletize debris. The supernatant was used for the FRAP assay, a colorimetric assay which measures the total antioxidant power of a solution by development of blue pigmentation (Benzie and Strain 1999). Samples (50  $\mu$ L) were placed in spectrophotometer cuvettes, and 1.5 mL of fresh FRAP reagent solution was added, fully mixing the solutions. The reaction was allowed to proceed for 6 min at room temperature to allow full development of the pigmentation. The absorbance of the reacted solution was read at 593 nm, and the value converted to FRAP  $\mu$ M equivalent using a previously determined standard curve (1000  $\mu$ M ascorbic acid = 2000  $\mu$ M FRAP). The data were scaled to the product weight used for each sample. The evaluations were performed three times in separate replications.

### Sensory Properties

The texture and color of the products were evaluated after treatment with radiation. Doses were chosen based on previously determined  $D_{10}$  values of *L. monocytogenes*. The  $\log_{10}$  reduction equivalencies vary, due to the varying  $D_{10}$  values obtained. The products were treated with 0 (control), 1.5 kGy (equivalent to 1.9-2.4  $\log_{10}$  reductions) or 3.2 kGy (equivalent to 4.2-5.1  $\log_{10}$  reductions). The

irradiation was conducted at 2C, as previously described. Material used for sensory evaluations was not given the radiation sterilization treatment.

Color values were taken with a Hunter Lab Miniscan XE meter (Hunter Laboratory, Reston, VA) to determine the brightness (L-value), greenness/redness (a-value) and blueness/yellowness (b-value) of the irradiated material. The test material was positioned so as to completely cover the lens surface. The meter was calibrated using white and black standard tiles. Illuminant D65, 10° standard observer, and a 2.5 cm port/viewing area were used. Four samples were analyzed for each product/dose combination. The experiment was performed three times in separate trials, using separately prepared sets of materials.

Maximum shear force values were taken with a TA-XT/2i texture analyzer running the TextureExpert 1.22 software package (Texture Technologies, Scarsdale, NY). The probe used for the soy and beef frankfurter products was a TA-7 Warner-Bratzler blade. The probe height calibration was set to a 20 mm return above the sample positioning plate height, total probe travel distance of 40 mm. For the tofu product, a TA-42 knife blade was used. Height calibration was 20 mm return above the sample positioning plate height, total travel distance of 18 mm. Ten samples were evaluated per product/dose combination. The experiment was performed three times, in separate trials.

### Statistical Analysis

The antioxidant power data and sensory characteristics data were evaluated using analysis of variance (ANOVA, SigmaStat v. 4.0, SPSS, Chicago, IL), using data pooled from the three replications, final population sizes of  $n=18$  and  $n=15$ , respectively. For the response of *L. monocytogenes* to irradiation, the significance of differences between the slopes for each product type was determined by analysis of covariance (ANCOVA) (Excel, Microsoft Corp. Redmond, WA), using data pooled from the three replications, final population size of  $n=9$ . The correlation of antioxidant power and  $D_{10}$  value was determined with the Pearson Product Moment Correlation (SigmaStat).

## RESULTS

Ionizing radiation reduced the viable population of *L. monocytogenes* in all products tested (Fig. 1). The radiation sensitivity of *L. monocytogenes* was significantly influenced by the composition of the suspending material (Table 2). The highest  $D_{10}$  value obtained, 0.761 kGy on Soy1, is 122% of the lowest  $D_{10}$  value obtained, 0.622 kGy on Beef and Tofu. For all products, the  $R^2$  value for the linear regressions was greater than 0.95.

The materials differed significantly in antioxidant strength (Table 3). The antioxidant power of Tofu was significantly lower than that of any other material.

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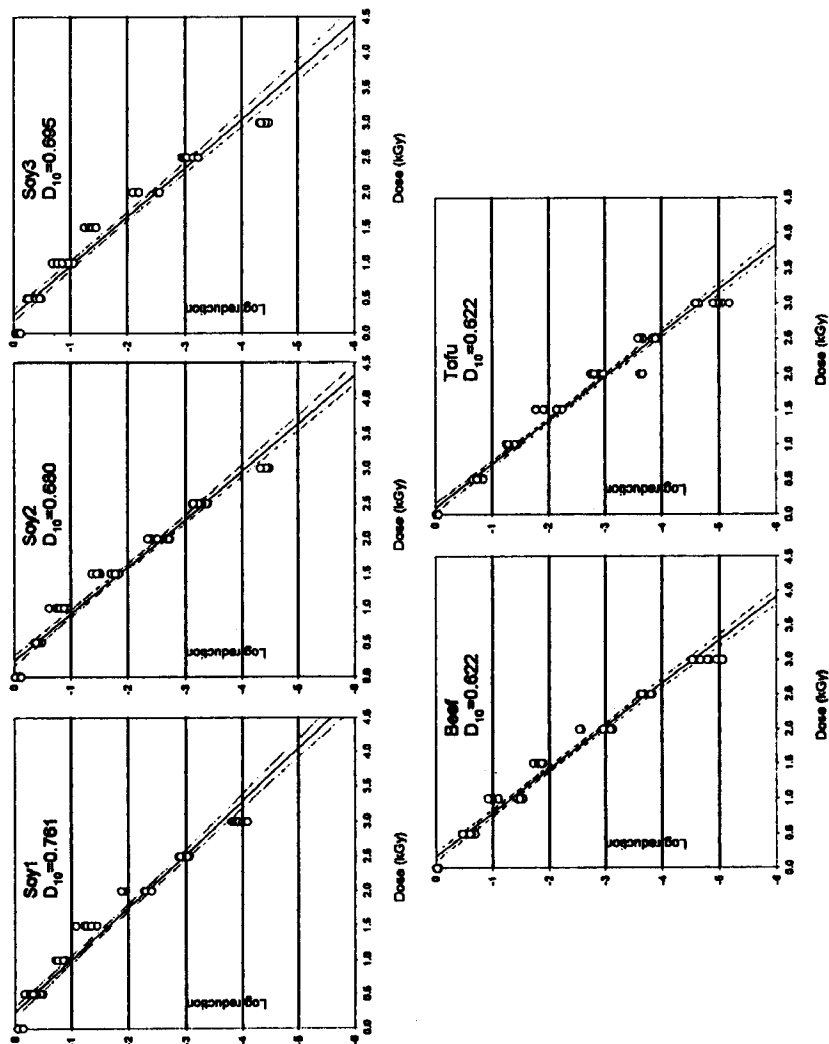


FIG. 1. RESPONSE TO IONIZING RADIATION OF *LISTERIA MONOCYTOGENES* INOCULATED ONTO SOY-BASED FRANKFURTERS, BEEF-BASED FRANKFURTERS AND TOFU  
Lines indicate linear regression with 95% confidence intervals.



TABLE 2.  
ANALYSIS OF COVARIANCE (ANCOVA) OF IRRADIATION  $D_{10}$  VALUES FOR *LISTERIA MONOCYTOGENES* ON FIVE COMMERCIAL READY-TO-EAT FOODS

	Beef	Soy1	Soy2	Soy3
Beef (0.622) <sup>1</sup>	-	-	-	-
Soy1 (0.761)	$P<0.05$	-	-	-
Soy2 (0.680)	$P<0.05$	$P<0.05$	-	-
Soy3 (0.659)	$P<0.05$	$P<0.05$	nsd <sup>2</sup>	-
Tofu (0.622)	nsd	$P<0.05$	$P<0.05$	$P<0.05$

<sup>1</sup>Product name is followed by irradiation  $D_{10}$  value, in kGy.

<sup>2</sup>nsd = not significantly different.

The soy-based frankfurters varied significantly, ranging from 840.4 to 3356.3  $\mu$ M FRAP. The antioxidant power of Beef was statistically similar to the more potent soy-based products (Table 3). The antioxidant power was not significantly correlated with the  $D_{10}$  values obtained: correlation coefficient = 0.62,  $P=0.26$ .

There were no significant ( $P<0.05$ ) differences in irradiated versus nonirradiated product with respect to brightness ("L") or blueness/yellowness ("b") (Table 3). Irradiation caused a significant decrease in redness in Beef and Soy2 and a significant increase in redness of Soy3 products, with no significant change in Soy1 or Tofu. Irradiation caused significant changes in maximum shear force in Beef and Soy1 products. For Soy1, irradiated frankfurters were significantly softer than nonirradiated. For Beef, the same general pattern was observed, with irradiated materials being softer than nonirradiated controls, but the data were more variable than for Soy1. In Soy2, Soy3 and Tofu, irradiation had no significant effect on the product texture.

## DISCUSSION

Ionizing radiation effectively reduced the population of *L. monocytogenes* on soy- and beef-based RTE foods in this study. These results show that the composition of commercial soy-based frankfurters can influence the radiation sensitivity of associated *L. monocytogenes*. The influence of the suspending food product on the radiation sensitivity of associated pathogenic bacteria has been shown with different meats (Thayer *et al.* 1995), different frankfurter formulations (Sommers and Thayer 2000) and types of vegetables (Niemira *et al.* 2002). The radiation  $D_{10}$  value obtained for this isolate of *L. monocytogenes* on beef

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TABLE 3.  
COLOR, TEXTURE AND ANTIOXIDANT POWER OF IRRADIATED SOY-BASED  
FRANKFURTERS, BEEF-BASED FRANKFURTERS AND TOFU

Product		Color			Texture (g) <sup>d</sup>	Antioxidant power ( $\mu$ M FRAP) <sup>e</sup>
		L <sup>a</sup>	a <sup>b</sup>	b <sup>c</sup>		
Beef	0.0kGy	51.0 a <sup>f</sup>	19.95 a	26.5 a	2210.1 a	2260.5 a
	1.5kGy	50.7 a	17.6 b	27.0 a	1763.2 b	
	3.2kGy	50.2 a	16.55 c	26.9 a	2016.9 c	
Soy1	0.0kGy	48.7 a	21.0 a	24.7 a	1202.4 a	3356.3 a
	1.5kGy	49.3 a	21.0 a	24.4 a	1118.0 b	
	3.2kGy	49.3 a	21.4 a	24.9 a	896.5 c	
Soy2	0.0kGy	58.0 a	14.1 a	30.7 a	584.7 a	840.4 b
	1.5kGy	58.1 a	13.9 ab	31.2 a	588.8 a	
	3.2kGy	58.1 a	13.7 b	30.6 a	520.9 a	
Soy3	0.0kGy	42.9 a	18.34 a	21.4 a	585.4 a	1195.7 ab
	1.5kGy	43.5 a	19.31 b	21.9 a	511.0 a	
	3.2kGy	42.7 a	19.62 b	22.1 a	503.7 a	
Tofu	0.0kGy	87.0 a	2.0 a	14.0 a	111.4 a	158.5 c
	1.5kGy	87.1 a	1.9 a	14.1 a	108.3 a	
	3.2kGy	87.2 a	1.9 a	14.1 a	96.6 a	

<sup>a</sup>Brightness: 0 = white, 100=black

<sup>b</sup>Green/red: negative "a" values indicate greenness; positive "a" values indicate redness

<sup>c</sup>Blue/yellow: negative "b" values indicate blueness; positive "b" values indicate yellowness

<sup>d</sup>Maximum shear force, in grams

<sup>e</sup>1000  $\mu$ M ascorbic acid = 2000  $\mu$ M FRAP

<sup>f</sup>Within each product and evaluation type, values for doses followed by the same letter are not significantly different.  $P < 0.05$ , analysis of variance, Tukey test.

frankfurters (0.622 kGy) is comparable to that previously obtained for the same isolate inoculated onto a similar beef frankfurter product (Sommers and Fan 2002).

Antioxidants have been shown to increase  $D_{10}$  values for bacteria suspended in solution by absorbing these radicals (Ho *et al.* 1995; Sharma *et al.* 2000). The potential protective role of antioxidants in irradiated foods is not fully understood. In studies of beef bologna (Sommers *et al.* 2001), orange juice (Niemira 2001) and lettuce (Niemira *et al.* 2002), the association of product antioxidant strength with

bacterial radiation sensitivity has been variable. Sommers *et al.* (2001) showed that relatively high antioxidant power, >5000  $\mu\text{M}$  FRAP, was necessary to influence the  $D_{10}$  of *L. monocytogenes* on beef bologna, a range higher than that observed in any of the products evaluated in this study. In the work presented herein, antioxidant power was not significantly correlated with the  $D_{10}$  values obtained. A primary ingredient for all of the soy-based products is SPC, a food additive known to enhance antioxidant power (Ho *et al.* 1995). The antioxidant power of the soy-based products was more variable than expected, and, in the case of tofu, lower than expected. Spices and flavorings can influence radiation  $D_{10}$  values (Sharma *et al.* 2000). The soy-based frankfurter products in this study are relatively complex, and were associated with significant variation in  $D_{10}$  obtained. *L. monocytogenes* showed a similar sensitivity when irradiated on the Beef frankfurter, a product containing a complex mixture of spices, flavorings and other ingredients, and Tofu, a relatively simple soy-based product. Although the composition of Tofu is relatively simple, inclusion of the acidulant gluconolactone could be responsible for the increased radiation sensitivity of *L. monocytogenes* in that product. Farlas and Andrassy (1993) found increased radiation sensitivity of microflora in chilled meat that contained gluconolactone. Sommers and Niemira (unpublished data) were able to increase the radiation sensitivity of *L. monocytogenes* surface-inoculated onto beef frankfurters by dipping the frankfurters in gluconolactone solutions. This observation, coupled with the lack of any meaningful association of  $D_{10}$  value and antioxidant power of the substrate, indicates that the effect of product composition on radiation sensitivity is complex and merits further investigation.

Radiation doses sufficient to cause  $\sim 2.2$  or  $\sim 4.8$   $\log_{10}$  reductions had a significant ( $P < 0.05$ ) and product-specific effect on sensory properties. The color of irradiated products was altered only with respect to redness. Beef and Soy2 frankfurters became less red with increasing dose, while Soy3 frankfurters became slightly more red. The loss of redness in beef frankfurters is consistent with the results obtained by Sommers *et al.* (2001), although those authors reported a radiation-associated loss of lightness and blueness color factors, changes not observed in the commercial product evaluated herein. The maximum shear force of Soy1 frankfurters decreased from 1202.4 g to 896.5 g at the highest dose, while the response of Beef frankfurters was variable. Thus, products which soften as a result of irradiation are not necessarily the same products which undergo color changes. The various aspects of product quality respond to irradiation differently, but each are dependant on product formulation.

These results show that irradiation can effectively reduce the viable population of *L. monocytogenes* on ready-to-eat processed food products. Depending on product formulation, the radiation dose required to achieve a 5- $\log_{10}$  reduction of the surface-inoculated *L. monocytogenes* ranged from 3.11 to 3.805 kGy. Sensory impact resulting from irradiation was dependant on the specific formulation of the product. The effect of specific product formulations on bacterial radiation sensitivity and product sensory response is a key area of future research.

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